Vector Surveillance in Southwest Virginia
June 2001- August 2002

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Summary

2001: L arval mosquito surveillance and mapping was performed throughout southwestern Virginia to determine the types and distribution of mosquito breeding habitats. Collecting sites were selected by referral from district employees and complaint calls. Collections were also done in areas affected by flooding. Containers and ground pools were found to be the most frequently encountered mosquito habitats. The most abundant vectors of WNV found in southwest Virginia were: ***C. pipiens, C. restuans, Ae. albopictus, Ae. vexans, and Oc. triseriatus.*** GPS coordinates were recorded at each site visited and entered into a database. The presence of Oc. japonicus was also discovered in Botetourt County. This is a recent import into the country and has not been reported from this area before.

Adult mosquitoes were also collected by CDC light traps and gravid traps in the New River and Alleghany districts. All mosquitoes were sorted, identified and then tested for virus. Of the over 1,500 mosquitoes tested, all were negative.

In addition to some larval surveillance in the Lenowisco Health District, an ovitrap study to develop a model for predicting mosquito abundance was done. Predictive maps of the distribution and abundance of ***Ae. albop***ictus and ***Oc. triseriatus*** were developed.

2002: Adult mosquito collection has been done at 7 sites in the New River District since June. In response to the discovery of virus-infected birds in Roanoke, collection sites have been established in the Alleghany Health District. Collections in both areas will continue until the onset of cold conditions kills off the mosquito population in the fall. All adult mosquitoes collected are being sorted, identified and pooled. They are being sent to Richmond for virus testing. Despite drought conditions, vector mosquitoes are abundant.

Recommendations

Productive mosquito habitats and vector mosquito populations are prevalent throughout southwestern Virginia. Despite the current drought conditions, mosquito populations are abundant. However, there is no baseline data on mosquito densities and abundance for southwest Virginia. Because of this, certain recommendations can be made:

- Active mosquito surveillance should be conducted in all areas each year.
    - By keeping yearly records, it can be determined when mosquito populations are high, low, or normal. This permits an estimate of the relative risk of virus transmission if it occurs in an area.
    - Mosquito surveillance is labor intensive and requires specialized training. Waiting until an outbreak occurs does not permit sufficient time to put together a response team.
    - Stable funding must be provided to develop and maintain a viable surveillance program. Without additional funding, my lab will be unable to do any additional surveillance work in 2003.
- Identification and mapping of mosquito breeding sites is an important component of mosquito surveillance. Many breeding sites can be eliminated. Those that can't be eliminated can often be treated with nonchemical anti-mosquito compounds (microbials and insect growth regulators) that have a low impact on non-target organisms.
- The development of predictive maps of mosquito abundance can make mosquito surveillance and control efforts more efficient. Key areas to concentrate on can be identified and emphasized in the surveillance design. This can reduce the time spent in the field and permit technicians to spend more of their day processing samples.
- Spraying for adult mosquitoes is costly, controversial, and not very effective. Sprays only knock down the portion of the population that is active on that particular night. Mosquitoes sheltered in vegetation are often protected and survive. It is best to emphasize mosquito control through treatment of breeding habitats.
- Other forms of surveillance should be considered. For instance, sentinel flocks can be used to detect the presence of virus in an area. Sentinel flocks have been more effective in the South for predicting WNV than in the Northeast.

1. Introduction

Mosquito surveillance, along with bird-based surveillance, should be the mainstay of regional surveillance programs for arboviruses, including West Nile virus (WNV) (CDC, 1999). An effective mosquito surveillance program provides an estimate of vector species abundance and distribution. This data is used to guide emergency control operations and to evaluate control methods. Laboratory testing of mosquitoes for virus infection provides information on the relative risk to humans and animals.

Surveillance entails the location, mapping, characterization of mosquito breeding habitats, including the use of Geographic Information System (GIS), of adult mosquitoes, submission of mosquitoes for laboratory testing for virus, and the determination of control options.

2. Location of Sampling Sites: 2001

Sampling was conducted in several counties throughout the southwest portion of the state (Figure 1). Emphasis was placed on collections in the New River, Alleghany, and Roanoke Health Districts because these areas are the most populous and would represent a higher health risk in the case of an outbreak of WNV.

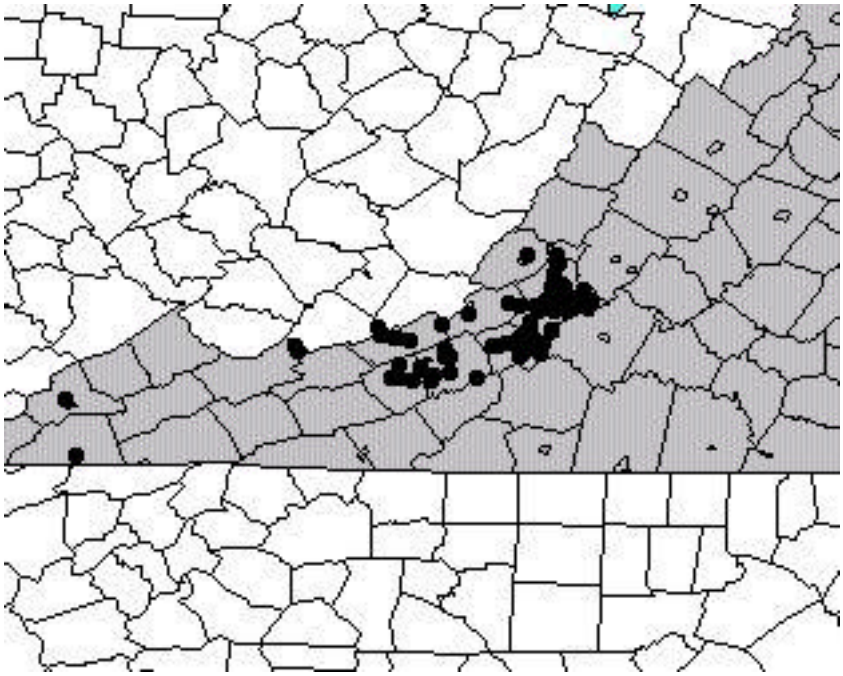

Figure 1. Sampling sites in southwest Virginia.

3. Collection Techniques

We employed several different collection techniques. Each method provides data that can be used in understanding the nature of the mosquito problem in a area so that a reasonable response can be designed in case of an outbreak. The types of collections made were:

- Larval collections
- Gravid traps
- Light traps
- Ovitraps

4. Larval Collections

Larval collections survey immature mosquitoes. This allows us to identify the particular breeding source of the mosquito species present in an area. It is thought that this is a more accurate measure of mosquito density in an area as compared to traps that collect adult mosquitoes. In locations like southwest Virginia where there is no baseline data, larval samples can be used to locate and map vector breeding sites. It can also be used to help determine appropriate locations for adult surveillance. In the study last summer, we used the data we collected for both purposes. Many of the sites visited were based on referrals by health district workers who had noticed potential sites while travelling through the counties as part of their other duties. We also investigated any complaint calls that came in to the districts.

At each site, larvae were collected and brought back to the lab at Virginia Tech to be identified to species. Also, GPS coordinates and digital photographs were taken at each location. This information was entered into a data base that can be consulted in the case of future problems in an area.

4.1 Larval Collection Results: Types of Habitats

The types and relative abundance of habitat types found are in Figure 2. The most common source of mosquitoes found was the category "Small Container." This includes items such as old tires, cans, bird baths and other materials capable of holding water. The next most common habitat encountered was in the category "Other." This consists of habitats such as ground pools, depressions in grassy areas that fill with water after a rain. These can be very productive in breeding mosquitoes (Fig. 3).

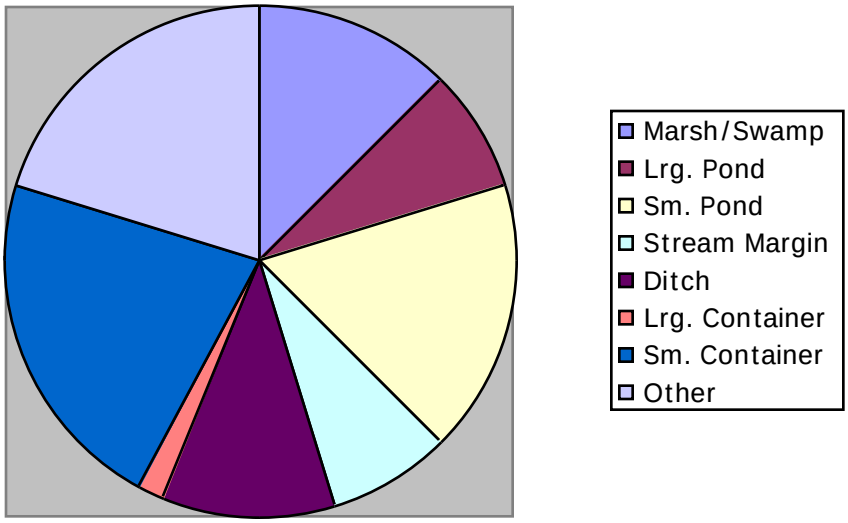

Figure 2. Breakdown of the types of larval habitats encountered in southwest Virginia.

Figure 3. Typical ground pool mosquito habitat.

4.2 Larval Collection Results: Data Sheets

Montgomery County
Blacksburg, VA

- Corner of Smithfield Plantation Dr. and Duck Pond Dr.
- GIS Coordinates: Alt. 649m, N 37°13.412', W 80°25.788'
- 16 *Culex restuans* larvae

June 6, 2001—This site included three to four large puddles located next to a gravel road on the Virginia Tech campus, an area of moderate traffic. The puddles, located in the grass, were approximately 2 meters in length and 3 inches in depth. Heavy rains caused the accumulation of water and the puddles did not last more than 8 days. A few trees surrounded the area producing shade over the puddles but no other vegetation was present. No water pollution was visible but when disturbed became cloudy with dirt. The larvae were moderately concentrated, yielding a total larvae/dip ratio of 2.00. Pupae were also present.

Figure 4. Example of data sheet produced for each sampling site.

4.2 Larval Collection Results: Mosquito Species

Several species were found to be prevalent throughout southwest Virginia.

- **Primary Vectors**
  - *Culex pipiens* and *Culex restuans*

    •***Cx. pipiens*** and ***Cx. restuans*** are the primary vectors responsible for maintaining WNV in an area. These mosquitoes are susceptible to WNV and readily utilize birds as hosts. However, they will feed on mammals, including humans, when these hosts are abundant. They are common in urban, suburban and rural locations and breed in a wide variety of habitats

including catch basins, ground pools, ditches, animal waste lagoons, and artificial containers.

- **Bridge Vectors: species that transmit WNV from wildlife to humans.**
  - *Aedes vexans*

    •Ae. vexans is an opportunistic feeder, willing to utilize a wide variety of hosts. It breeds in many different kinds of habitats including temporary and semi-permanent ground pools, floodplains, ditches and grassy rain pools.
  - *Ochlerotatus triseriatus*

    •***Oe. triseriatus*** is a potential bridge vector of WNV and is the primary vector of La Crosse encephalitis virus (LACV), a disease emerging in the southwest portion of Virginia. LACV causes encephalitic disease primarily in children. The natural habitat of ***Oe. triseriatus*** is treeholes, but it readily utilizes man-made containers such as tires and cans.
  - *Aedes albopictus*

    •***Ae. albopictus*** is a container-breeder, using habitats similar to ***Oe. triseriatus***. Although it will feed on many different hosts, it is an avid man-biter. Unlike many mosquitoes, it is very active during the day. This species is especially prevalent in urban and suburban habitats.

4.3 Larval Collection Results: New Mosquito Found

During the surveillance program in 2001, it was discovered that Ochlerotatus japonicus is established in the Eagle Rock area. This mosquito is newly introduced into the U.S., having been found first in NY and NJ in 1998. It has continued to increase its distribution since then. This mosquito is a potential bridge vector of WNV. It was found at 4 sites in Botetourt County. The spread of this mosquito in southwest Virginia should be monitored.

5. Adult Mosquito Collections

Because it is the adult female mosquito that transmits disease, many surveillance techniques have been devised to collect adult mosquitoes. This permits the determination of species in an area, the abundance of mosquitoes, and the mosquitoes collected can be tested in the lab for the presence of virus. Adult mosquitoes were collected in gravid traps and light traps. In 2001, based on information gained during the larval surveillance, trapping was done at 6 locations beginning in August and continuing until the mosquito population declined in late September. The location of these sites is shown in Fig. 5.

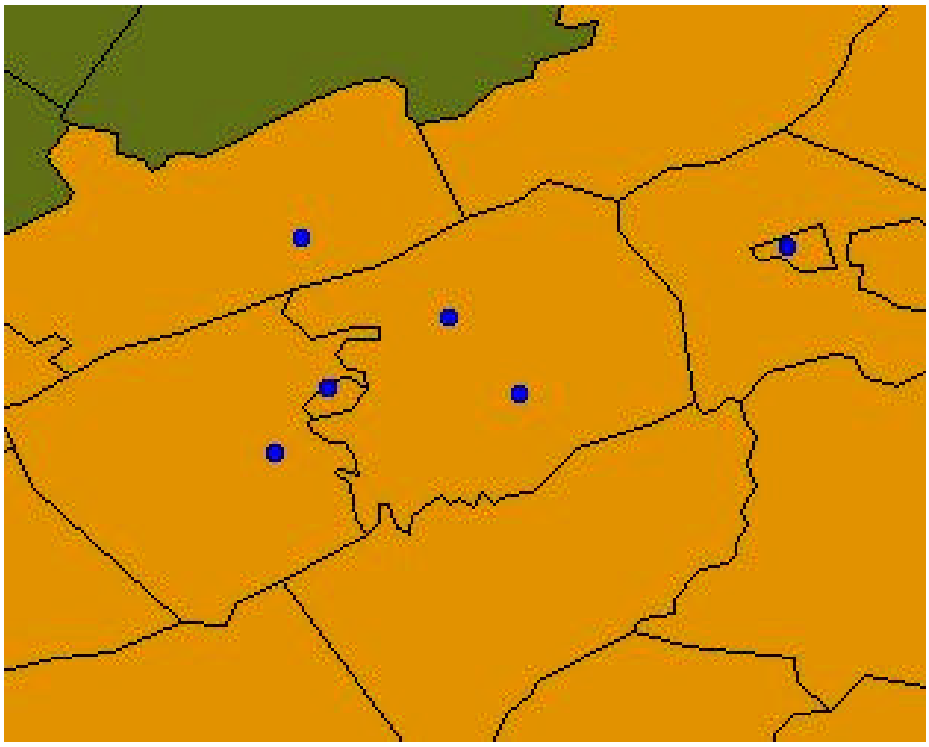

Figure 6. Location of adult collecting sites in 2001.

5.1 Adult Mosquito Collections: Gravid Traps

This trap samples female Culex mosquitoes as they come to oviposit. Therefore, it is selective for females that have already taken at least one bloodmeal. There is a higher probability of collecting virus-infected mosquitoes in a gravid trap than in a light trap.

5.2 Adult Mosquito Collections: Gravid Trap Results

Significant differences were found between locations in terms of numbers of mosquitoes collected. The Blacksburg site was the most productive. The primary vectors, ***Cx. pipiens*** and ***Cx. restuans***, were the most common species collected in the gravid traps.

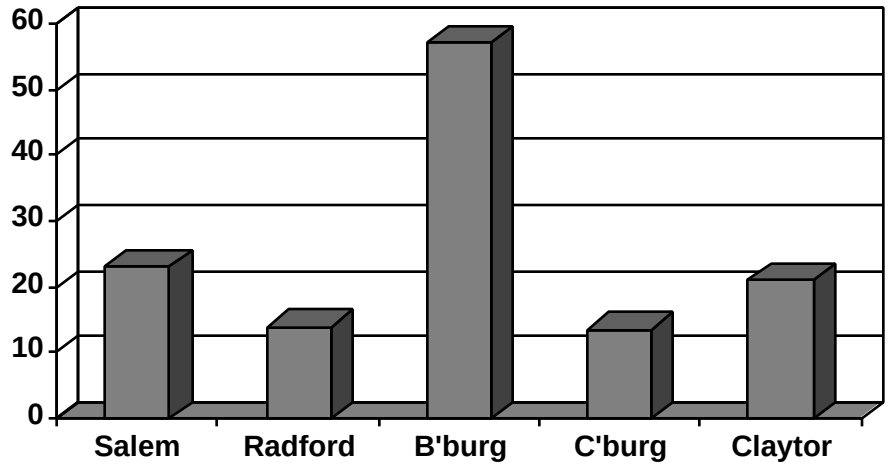

Figure 6. Mean number of mosquitoes collected per trap night.

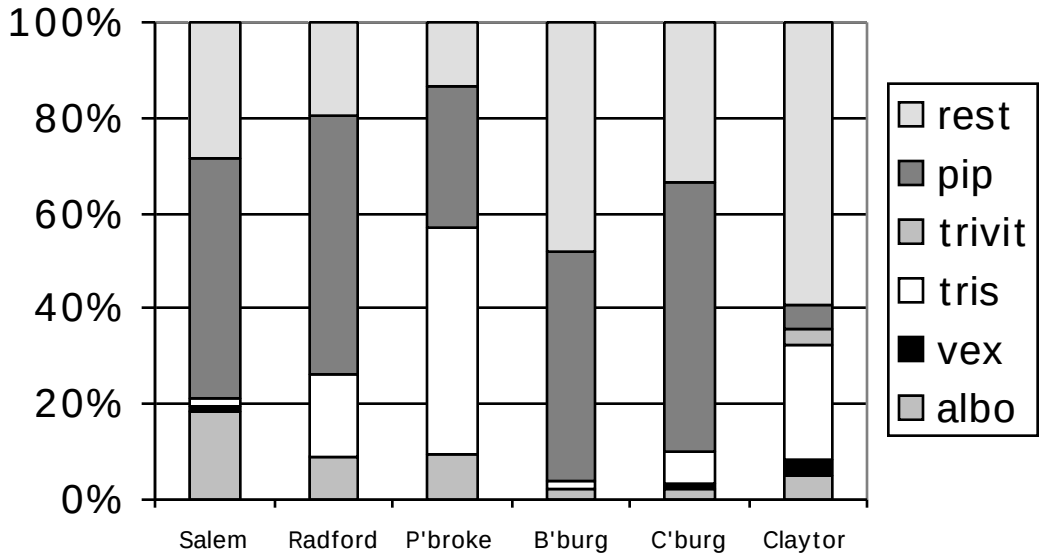

Figure 7. Species composition of mosquitoes collected by gravid traps.

5.3 Adult Mosquito Collections: CDC Light Traps

These traps are considered the standard tool for arbovirus surveillance. The CDC trap is light, portable and uses a 6-volt battery so it doesn't require permanent installation. Baiting the trap with CO_2 (using dry ice) as an attractant will increase both the number of mosquitoes and the range of species collected as compared to traps using light as the sole attractant. Mosquitoes are collected live in a net so virus isolation can be performed. Environmental conditions such as temperature and humidity can significantly affect the catch. Another disadvantage is that in some areas it is may difficult to obtain dry ice. The portability of the traps make them the best choice for regions where mosquito surveillance has not been done routinely because they can be easily moved from one site to another.

5.4 Adult Mosquito Collections: CDC Light Trap Results

The results of the light trap collections yielded a different pattern. The site in Christiansburg was the most productive. Also, although ***Cx. pipiens*** and ***Cx. restuans*** were collected, the most common species was ***Ae. vexans***. ***Oc. triseriatus*** was also frequently collected. These differences were expected; gravid traps and light traps preferentially collect different species. For this reason, it is best to include both types of traps in a surveillance program.

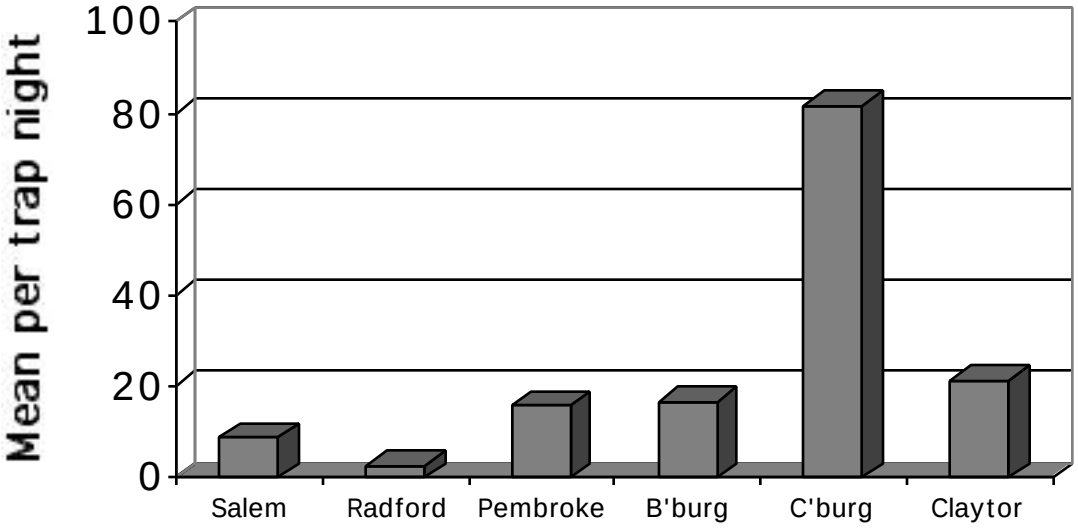

Figure 8. Mean number of mosquitoes collected per trap night.

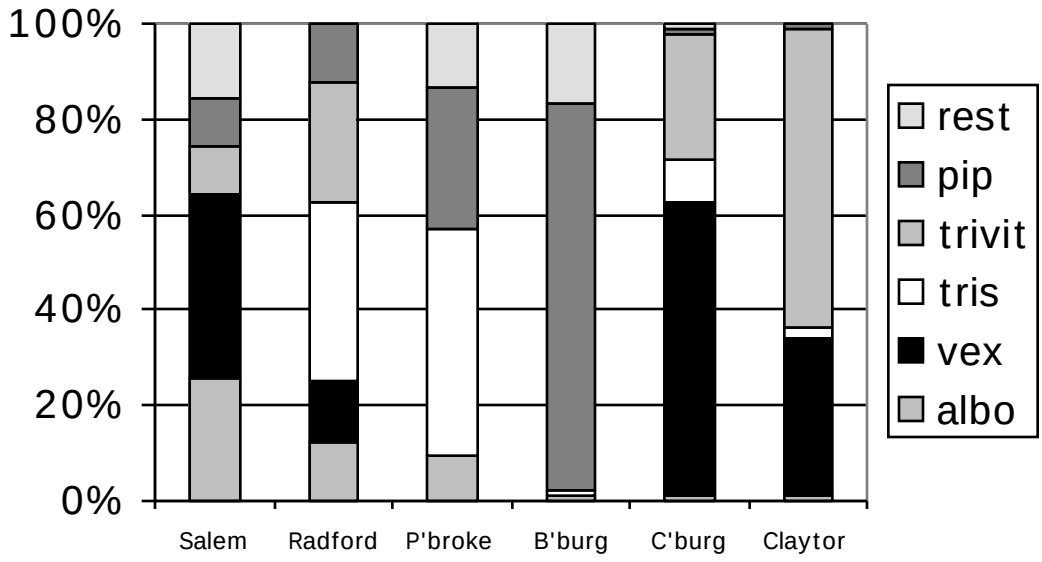

Figure 9. Species composition of mosquitoes collected by CDC light traps.

6. Surveillance by Ovitraps

Ovitraps are simply black plastic cups partially filled with water and equipped with strips of textured paper. A female mosquito enters the cup and lays her eggs on the moist paper. These papers are then collected and brought into the lab. The eggs can be hatched and the mosquitoes raised so that virus isolation can be performed. This is the most effective way to monitor *Oc. triseriatus* and *Ae. albopictus* populations because these species are poorly attracted to light traps or gravid traps.

6.1 Distribution of Vectors in Lenowisco Health District

In work done earlier, it was found that both ***Oc.triseriatus*** and ***Ae. albopictus*** were widespread and abundant in Lee, Scott and Wise counties (Fig. 10). Because these mosquitoes are container-breeders, they are difficult to control. However, they are important vectors of La Crosse encephalitis and bridge vectors of WNV.

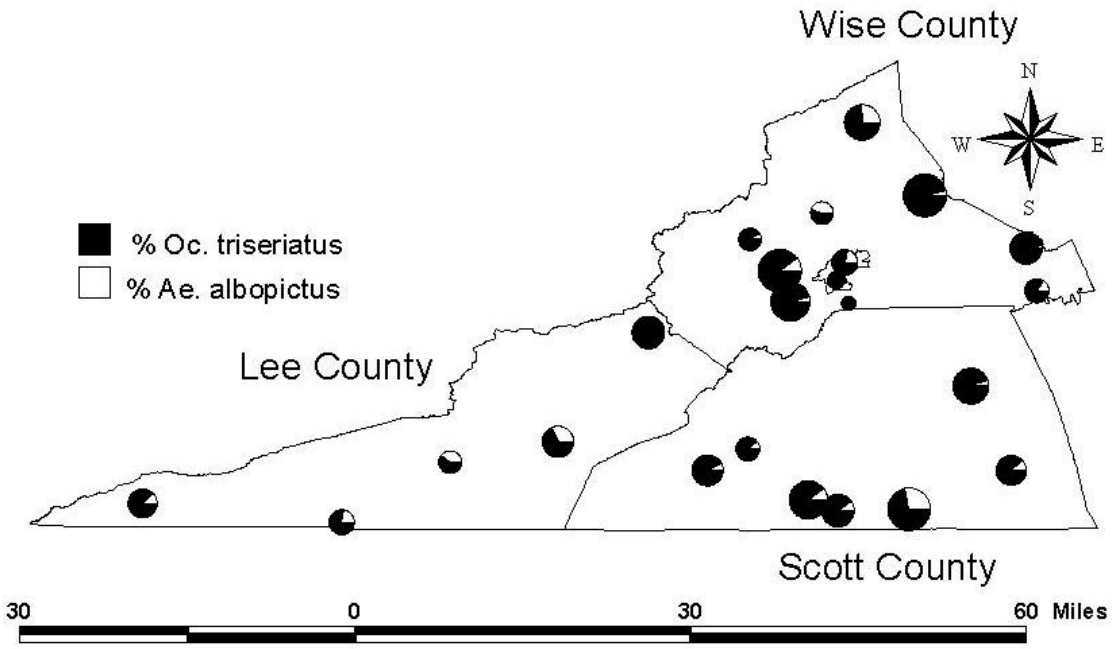

Figure 10. Distribution and abundance of vector mosquitoes.

6.2 Using Mosquito Surveillance and Landcover Data to Develop a Predictive Model of Mosquito Distribution

There are many obstacles to developing a mosquito surveillance program. First, trapping for adult mosquitoes is very labor intensive and requires a training program to develop an expertise in mosquito identification in the workers. Also, light traps and gravid traps are typically placed in the field in the afternoon and picked up the following morning. When dealing with a large geographic area, the travel time required is significant and removes needed technicians from the lab. One way to make surveillance more efficient is to develop a predictive model of mosquito distribution and abundance using satellite imagery that will allow workers to identify key areas for study. We have attempted to develop a model for ***Ae. albopictus*** and ***Oc. triseriatus*** in Wise County.

Using Landsat imagery, a landcover map for the county was produced (Fig. 11). It was found that there were 5 main habitat types: forest, urban/residential, shrub and brush rangeland, herbaceous rangeland, and barren. Mosquito eggs were collected from 160 sites over a 16-week period in 2000 and from 40 sites over a 3-week period in 2001. The sites represented the various habitat types. A preliminary model was developed with the 2000 data and the 2001 data is being used to refine the model further. It was found that method can be used to develop predictive maps of mosquito abundance (Fig. 12). This method can be utilized for other species by substituting the kind of data collected. For instance, gravid traps could be used to develop a model for ***Culex*** distribution. Once a predictive map is produced, mosquito surveillance and control efforts can be concentrated in areas where the species of interest is most likely to be abundant, conserving resources.

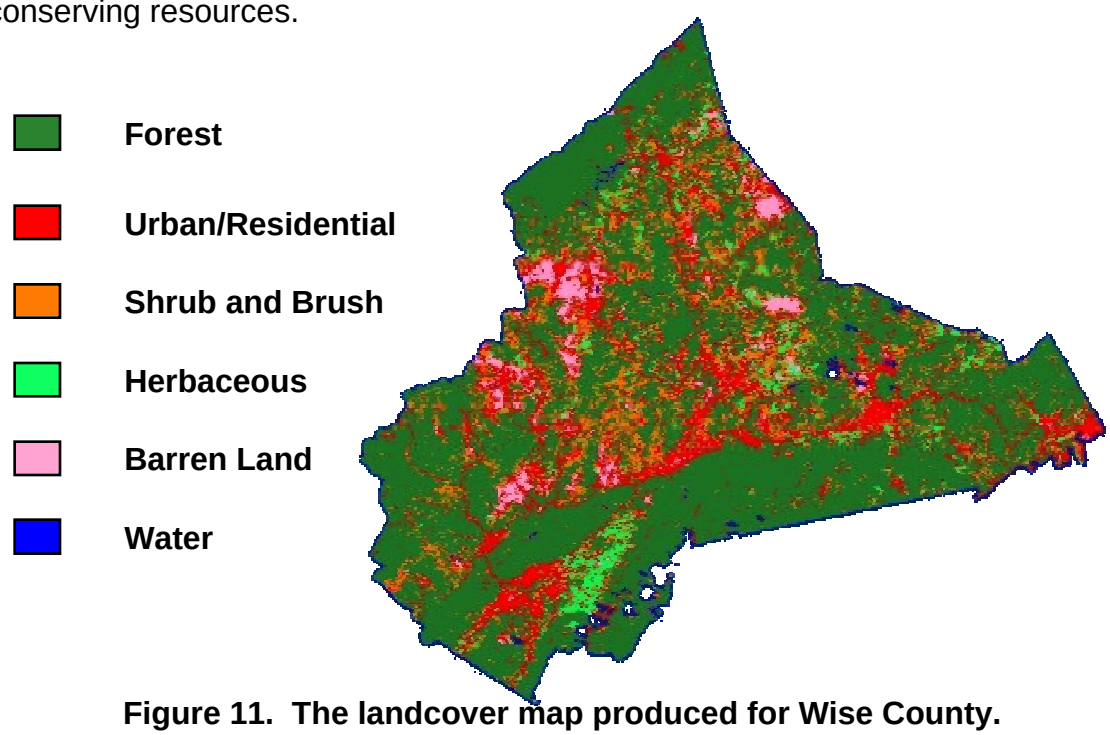

Figure 11. The landcover map produced for Wise County.

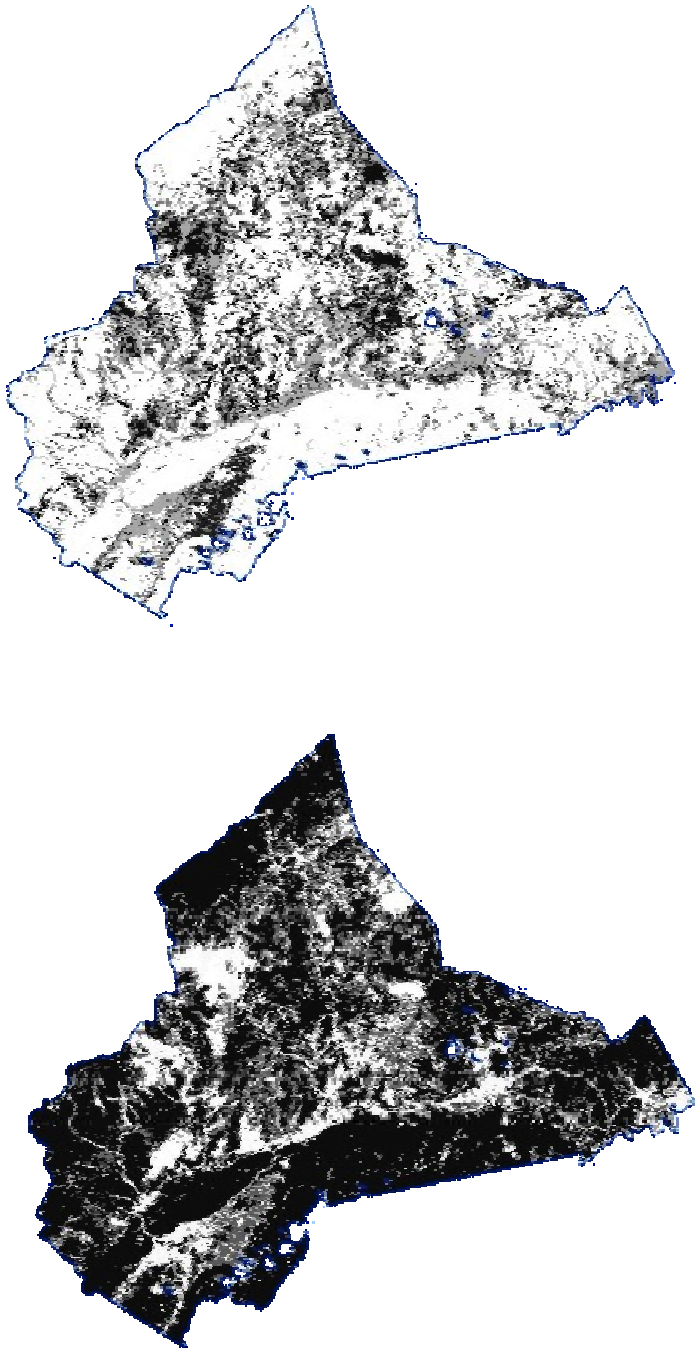

Figure 12. Predictive maps of mosquito abundance in Wise County. The top map is *Oc. triseriatus* and the bottom map is *Ae. albopictus*. Areas in white indicate locations of highest abundance. *Oc. triseriatus* was found to be widespread and most abundant in forested areas. *Ae. albopictus* was most prevalent in urban/residential areas.

7. Virus Isolation

All adult mosquitoes collected in 2001 were tested for the presence of virus in the lab at Virginia Tech. Over 1,500 mosquitoes were tested and all were negative. This result was not suprising given the absence of any infected birds in the areas where mosquitoes were collected.

8. Personnel:

2001. Virginia Tech: Bryan Jackson, graduate student, Courtney Brown, Monica Goyanko, Stephen Ciesielski (volunteer). Barbara Kolb and numerous volunteers assisted in the collections in Botetourt County.

2002. Virginia Tech: Bryan Jackson, graduate student, Matthew Roller (1/2 time), Nathan Young (1/2 time), Devin Grimm (volunteer). Barbara Kolb will again assist in collections this fall for ***Oc. japonicus***.

9. Funding Sources:

Virginia Department of Health
Alleghany Health District
Cumberland Health District
Lenowisco Health District
New River Health District